

Title (en)  
NOVEL GENE TARGETING METHOD

Title (de)  
NEUARTIGES GEN-TARGETING-VERFAHREN

Title (fr)  
NOUVEAU PROCÉDÉ DE CIBLAGE DE GÈNE

Publication  
**EP 3290518 A4 20181205 (EN)**

Application  
**EP 16785983 A 20160429**

Priority  
• CN 201510220631 A 20150430  
• CN 2016080785 W 20160429

Abstract (en)  
[origin: EP3290518A1] Provided are a novel two-step gene targeting method and a nucleotide construct for gene targeting. The method can improve the gene targeting efficiency and accurately identify a target gene knock-out mutant. The method of the present invention comprises: firstly, efficiently replacing a target gene in a genome with a targeting box by homologous recombination, the targeting box consisting of a target gene activity variant, a marker gene and site-specific recombination sites; and secondly, resecting the targeting box by recombinase, leaving a site-specific recombination site on the target gene to generate a target gene knock-out mutant, and removing a recombinase expression vector from the knock-out mutant by using a counter selection marker in the recombinase expression vector.

IPC 8 full level  
**C12N 15/11** (2006.01); **C12N 1/16** (2006.01); **C12N 15/10** (2006.01); **C12P 21/00** (2006.01)

CPC (source: EP US)  
**C12N 1/16** (2013.01 - EP); **C12N 9/1241** (2013.01 - EP US); **C12N 15/11** (2013.01 - EP US); **C12N 15/902** (2013.01 - EP);  
**C12N 15/905** (2013.01 - EP US); **C12P 21/00** (2013.01 - EP); **C12N 2800/30** (2013.01 - EP)

Citation (search report)  
• [XY] HEANEY JASON D. ET AL.: "Tissue-specific expression of a BAC transgene targeted to the Hprt locus in mouse embryonic stem cells", GENOMICS, vol. 83, no. 6, 2004, pages 1072 - 1082, XP029302978, ISSN: 0888-7543, DOI: 10.1016/J.YGENO.2003.12.015  
• [XY] HERNANDEZ DIANA ET AL.: "Deletion of genes from the mouse genome using Cre/loxP technology", METHODS IN MOLECULAR BIOLOGY (CLIFTON, N.J.) 2006, vol. 320, 2006, pages 307 - 319, XP009508620, ISSN: 1064-3745  
• [Y] JOHANSSON B. AND HAHN-HÄGERDAL B.: "Overproduction of pentose phosphate pathway enzymes using a new CRE-loxP expression vector for repeated genomic integration in Saccharomyces cerevisiae", YEAST, JOHN WILEY & SONS LTD, GB, vol. 19, no. 3, 1 February 2002 (2002-02-01), pages 225 - 231, XP002399562, ISSN: 0749-503X, DOI: 10.1002/YEA.833  
• [A] STEPHAN J. ET AL.: "Consecutive gene deletions in Mycobacterium smegmatis using the yeast FLP recombinase", GENE, ELSEVIER, AMSTERDAM, NL, vol. 343, no. 1, 8 December 2004 (2004-12-08), pages 181 - 190, XP004653653, ISSN: 0378-1119, DOI: 10.1016/J.GENE.2004.08.028  
• See references of WO 2016173555A1

Designated contracting state (EPC)  
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

DOCDB simple family (publication)  
**EP 3290518 A1 20180307**; **EP 3290518 A4 20181205**; CN 106191041 A 20161207; CN 106191041 B 20201229; US 11299754 B2 20220412; US 2018355381 A1 20181213; WO 2016173555 A1 20161103

DOCDB simple family (application)  
**EP 16785983 A 20160429**; CN 201510220631 A 20150430; CN 2016080785 W 20160429; US 201615570517 A 20160429